PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/009321	19.08.2004	20.08.2003

International Patent Classification (IPC) or both national classification and IPC
C12P21/02

Applicant
SANDOZ AG

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/009321

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	7,8,13
	No:	Claims	1-6,9-12,14-23
Inventive step (IS)	Yes:	Claims	7,8,13
	No:	Claims	1-6,9-12,14-23
Industrial applicability (IA)	Yes:	Claims	1-23
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement (Continuation)

2.1 CITATIONS

Reference is made to the following documents:

D1: HART R A ET AL: "Large scale, in situ isolation of periplasmic IGF-I from E. coli" BIO/TECHNOLOGY, vol. 12, November 1994, pages 1113-1117

D2: EP-A-0 177 343 (GENENTECH INC) 9 April 1986

D3: WO 03/004599 A (PANCER ZEEV ; PELEG YOAV (IL); INSIGHT STRATEGY & MARKETING L (IL)) 16 January 2003

2.2 NOVELTY (Art. 33(2) PCT)

2.2.1 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of **claims 1-6, 9-12, and 14-23** is not new in the sense of Article 33(2) PCT.

2.2.2 **D1** discloses a process for the preparation of recombinant IGF-I produced by *Escherichia coli*, wherein it is secreted into the periplasm, whereby further processing of the fermentation harvest broth is interrupted by a step of solubilisation (cf., e.g., page 1116 right-hand column paragraph 'IGF-I in situ solubilization'), falling within the terms of **claims 1-3, 6, 9, 16-21 and 23**.

2.2.3 **D2** discloses a process for the preparation of recombinant human growth hormone by *E. coli*, wherein it is secreted into the periplasm, whereby further processing of the fermentation harvest broth is interrupted by a step of killing the cells (cf., e.g., example 8, and claims 13 and 15), falling within the terms of **claims 1, 6, 9-12 and 16-23**.

2.2.4 **D3** discloses a process for the preparation of recombinant human growth hormone by *E. coli*, wherein it is secreted into the periplasm, whereby further

processing of the fermentation harvest broth is interrupted by storage of cells at -20 °C (cf. example 3), falling within the terms of **claims 1-5, 14, 15, and 17-23**.

2.2.5 The combination of features of the dependent **claims 7, 8 and 13** with the features of **claim 1** to which they refer is not known from the available prior art. The subject-matter of these claims can therefore be regarded as new in respect of the prior art as defined in the regulations (Rule 64(1)-(3) PCT).

2.3 INVENTIVE STEP (Art. 33(3) PCT)

2.3.1 **D2** is regarded as being the closest prior art to the subject-matter of **claim 1** and discloses a method for recovering a recombinant protein, preferably recombinant human growth hormone, from the periplasmic space of a bacterial cell, preferably *E. coli*, comprising the steps of growing the cells whereby the protein is secreted in the periplasm, killing the cells, and recovering the protein of interest from the cells by a freeze-thaw procedure (cf., example 8, claims 13 and 15). The problem solved by **D2** is the provision of an improved method to recover periplasmic proteins, preferably eukaryotic proteins produced in bacterial hosts, preferably, human growth hormone (cf. page 6 line 33 - page 7 line 12). The step of killing the cells prior to extraction is said to approximately double the product protein recovery without reducing the purity of the product protein in the recovered supernatants (cf. page 21 line 24-26). The disclosure of **D2** renders the subject-matter of **claims 1, 6, 9-12 and 16-23** not novel, and consequently not inventive.

2.3.2 Similarly, **D1** and **D3** can be regarded as closest prior art, rendering the subject-matter of **claims 1-3, 6, 9, 16-21 and 23** and of **claims 1-5, 14, 15, and 17-23**, respectively, not novel and consequently not inventive, either.

2.3.3 The subject-matter of **claims 7, 8 and 13** in combination with the features of **claim 1** to which they refer, can be regarded as inventive, as they provide solutions to the problem of providing an improved process for the isolation of recombinant proteins expressed in the periplasm of bacterial cells, which are not obvious to the skilled person.

2.4 INDUSTRIAL APPLICABILITY (Art. 33(4) PCT)

2.4.1 The subject-matter of **claims 1-23** satisfies the criterion set forth in Art. 33(4) PCT in conjunction with Rule 5(vi) PCT with respect to industrial applicability.

Re Item VIII
Certain observations on the international application (Continuation)

1 CLARITY (Art. 6 PCT)

1.1 The use of broad terms in **claim 1** renders the scope of the claim unclear, as it is not clear what may be encompassed by terms such as 'further processing of the fermentation harvest broth' and 'maintaining it under defined conditions'.

1.2 The subject-matter of **claim 23** is neither clear nor concise, as it seeks to encompass the whole description in a claim. Such claims are not allowable.

2 SUPPORT (Art. 6 PCT)

2.1 The solution as presented in the current application, particularly referencing to example 1, appears to go against a general prejudice in the field that lengthening of the isolation procedure will result in a *de*crease in the production of recombinant proteins. For this, ample evidence is present in the literature, part of which has been referred to by the applicant in the application. In contrast, based upon the finding that in the case presented in example 1 the production of a recombinant Fab' with specificity for TNFalpha is *in*creased rather than *de*creased when further processing is interrupted before extraction, a broad **claim 1** has been formulated. It is pointed out that current examples 2 and 3 represent mere assertions that the rhGH and rIFN-alpha 2B extraction yields can be increased by an interruption step.

2.2 There is sufficient reason to assert that a broad claim such as **claim 1** is not supported over the whole of its scope, and that the invention is not practicable

for each and every recombinant protein secreted into the periplasm of a bacterial cell. From the prior art, e.g., as indicated by the applicant in the application, it is apparent to the skilled person that the problem which is dealt with in the current application is not solved for all recombinant proteins by the means offered in the application and referred to in **claim 1**. It is to be expected that the technical effect of increasing the extraction yield of a protein produced in the periplasm of a bacterial cell by including an interruption step prior to extraction, will not be achieved over the whole of the scope of **claim 1**. Henceforth, a lack of support for **claim 1** is noted, contrary to Art. 6 PCT.

2.3 In line with this reasoning, also the subject-matter of all dependent claims is considered to be unsufficiently supported over the width of the claims.